CD8⁺ T-cell immunity plays an important role in protection against intracellular infections. Earlier studies have shown that CD4⁺ T-cell help was needed for launching in vivo CD8⁺ T-cell activity against these pathogens and tumors. However, recently, CD4⁺ T-cell-independent CD8 responses during several microbial infections including those with Toxoplasma gondii have been described, although the mechanism is not understood. We now demonstrate that, in the absence of CD4⁺ T cells, T. gondii-infected mice exhibit an extended NK cell response, which is mediated by continued interleukin-12 (IL-12) secretion. This prolonged NK cell response is critical for priming parasite-specific CD8⁺ T-cell immunity. Depletion of NK cells inhibited the generation of CD8⁺ T-cell immunity in CD4⁻/⁻ mice. Similarly neutralization of IL-12 reduces NK cell numbers in infected animals and leads to the down-regulation of CD8⁺ T-cell immunity against T. gondii. Adoptive transfer of NK cells into the IL-12-depleted animals restored their CD8⁺ T-cell immune response, and animals exhibited reduced mortality. NK cell gamma interferon was essential for cytotoxic T-lymphocyte priming. Our studies for the first time demonstrate that, in the absence of CD4⁺ T cells, NK cells can play an important role in induction of primary CD8⁺ T-cell immunity against an intracellular infection. These observations have therapeutic implications for immunocompromised individuals, including those with human immunodeficiency virus infection.

The role of CD4⁺ T cells in the development of CD8⁺ T-cell effector responses against microbial pathogens has been studied (36, 39). Although the presence of CD4⁺ T cells is absolutely essential for the induction of CD8⁺ T-cell immunity against certain infections (7, 27, 34), CD4-independent responses to several infectious diseases and tumors have also been observed (37, 40, 46). CD8⁺ T-cell immunity against lymphocytic choriomeningitis virus was severely compromised in mice lacking CD4⁺ T cells (28). Coordinated interaction between CD4⁺ and CD8⁺ T cells was required to resolve an infection with the intracellular bacterium Listeria monocytogenes (43). Conversely, absence of CD4⁺ T cells does not affect the development of CD8⁺ T-cell responses against Encephalitozoon cuniculi (30). Seemingly normal CD8⁺ T-cell immunity against Plasmodium yoelii is initiated in CD4⁺ T-cell-depleted animals (4).

Protective immunity against Toxoplasma gondii has been reported to be dependent on both CD4⁺ and CD8⁺ T cells (8). While CD8⁺ T cells are essential for effector immunity against the parasite, CD4⁺ T cells play an important synergistic role by helping maintain a robust CD8⁺ T-cell response. Recent studies from our laboratory have demonstrated that, although long-term CD8⁺ T-cell immunity cannot be maintained in the absence of CD4⁺ T cells, initiation of this response in CD4⁻/⁻ mice is comparable to parental wild-type animals (5). As a consequence, CD4⁻/⁻ mice are able to resolve acute infection and do not exhibit increased mortality in response to the challenge. Thus T. gondii falls in a group of pathogens in which CD8⁺ T-cell immunity can be induced in a T-helper-cell-independent manner. Although T-helper-independent induction of CD8⁺ T-cell response in T. gondii and other pathogens has been observed (4, 40), the mechanism of this response has not been identified. Factors responsible for the development of CD8⁺ T-cell immunity in the absence of conventional CD4⁺ T cells need identification.

MATERIALS AND METHODS

Mice, parasites, and infection. Six- to eight-week-old female CD4⁻/⁻ and wild-type C57BL/6 mice were obtained from Jackson Laboratory (Bar Harbor, ME). Mice were maintained in a specific-pathogen-free environment in the Animal Research Facility at Louisiana State University Health Sciences Center (New Orleans, LA). All experiments were performed in accordance with Institutional Animal Care and Use Committee regulations. Mice were challenged perorally with 20 cysts of T. gondii strain 76K (provided by Daniel Bout, Tours, France).

Quantization of parasite load. Quantization of parasite burden on the tissues (spleen, liver, and brain) was performed at day 7 postinfection (p.i.) by quantitative PCR as previously described (20). DNA was isolated from tissues with the Qiamp tissue kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. Parasite DNA was amplified with primers specific for a 35-fold-repetitive sequence of the B1 gene (5'-TCTTTAAAGCGTTCGTGGTC-3' and 5'-GGAACTGCATCCGTTCATGAG-3', amplified along with parasite DNA. Amplification was performed using a 50-μl reaction mixture containing 1.24 U AmpliTaq DNA polymerase; 1× PCR buffer (Promega, Madison, WI); 0.2 mM each of dGTP, dATP, dTTP, and dCTP; and 0.4 mM of each B1 primer. For each reaction, a known amount of DNA from the tissues was amplified with various amounts of the internal standard. The levels of parasite load were estimated by comparison to the internal controls. PCR was performed under the same conditions using a known number of parasites. The level of internal control was calculated per parasite (23).
NK cell response and depletion. NK cell numbers were determined at days 0, 4, 7, 14, 21, and 28 p.i. by fluorescence-activated cell sorter (FACS) analysis. Spleens were disrupted between glass slides, and red blood cells were removed by lysis with red blood cell lysis buffer (Sigma-Aldrich, St. Louis, MO). Spleenocytes were stained with fluorescein isothiocyanate (FITC)-labeled anti-NK1.1 (BD Biosciences, San Diego, CA) and assayed for NK cell expression by FACS analysis. Acquisition of the data was done on FACS Vantage SE (BD Biosciences, Franklin Lakes, NJ). Results were analyzed with CellQuest software (BD Biosciences). For NK cell depletion, mice were treated with 50 μl anti-asialo-GM1 antibody (Wako Chemicals, Richmond, VA) starting 1 day prior to infection and thrice weekly thereafter, until the termination of the experiment. The control mice were administered equivalent amounts of rabbit immunoglobulin G (IgG) isotype control antibody (Sigma-Aldrich, St. Louis, MO). Anti-asialo-GM1 treatment led to >95% elimination of NK cells as determined by FACS analysis.

pCTL frequency analysis. Purified CD8+ T cells (95% pure), isolated by magnetic separation (Miltenyi Biotech, Auburn, CA) were cultured in a limiting dilution assay in the presence of irradiated parasites and antigen-presenting cells as described earlier (19). After 1 week in culture the precursor cytotoxic T-lymphocyte (pCTL) frequency of the effectors was determined by a standard protocol of our laboratory (22).

IFN-γ production by CD8+ T cells. Gamma interferon (IFN-γ) production by CD8+ T cells was evaluated by intracellular staining as previously described (22). A whole spleen cell population was prepared as mentioned above and cytokine analysis performed. Briefly, spleenocytes were stimulated with 10 ng/ml phorbol myristate acetate (Sigma), 500 ng/ml of ionomycin (Sigma-Aldrich), and 2 μM monomeric (Golgistop; BD PharMingen). Cultures were incubated for 4 h at 37°C in 5% CO2. After incubation, cells were washed with phosphate-buffered saline (PBS) and 1% fetal calf serum and stained with anti-CD8α conjugated with FITC (eBioscience) for 30 min at 4°C. Intracellular staining was performed using the Cytofix/CytoPerm kit (BD PharMingen) according to the manufacturer’s instructions. Intracellular staining was then performed using anti-IFN-γ or an isotype-matched control antibody conjugated with phycoerythrin (PE; BD PharMingen). Samples were resuspended in PBS containing 1% formaldehyde and analyzed by FACS.

Serological levels of cytokines. Sera from infected CD4−/− mice and wild-type mice were collected at different time points p.i. and assayed for cytokines by using enzyme-linked immunosorbent assay kits for interleukin-12 (IL-12) (Biorad, San Diego, CA) and IL-10 (R&D Systems, Minneapolis, MN) according to the manufacturers’ instructions. IL-12 depletion. Mice were depleted of IL-12 by administering rat anti-mouse IL-12 as previously described (16). Mice were given 0.5 mg doses of anti-IL-12 antibody on days 0, 1, and 2 p.i. for early depletion or on days 3, 4, and 5 p.i. for late depletion. Control animals were given the same amount of a rat IgG isotype control (Sigma-Aldrich).

Adoptive transfer of NK cells. NK cells were isolated by positive selection via magnetically assisted cell sorting (MACS; Miltenyi Biotech) according to the manufacturer’s instructions. Cells were labeled with biotinylated anti-CD90 and anti-CD19 (eBioscience) and antibiotin microbeads (Miltenyi Biotech). NK cells were isolated by positive selection via MACS. Purified cells (90% pure) were then depleted of NK cells by FITC-conjugated anti-NK1.1 (PharMingen) and passed through a flow cytometer. Purified NK cells (105; 99% pure) from either C57BL/6 or IFN-γ−/− mice were injected intravenously to CD4−/− animals at day 5 p.i.

Statistical analysis. Statistical analysis of the data was performed by Student’s t test (31).

RESULTS

CD4−/− mice develop a strong NK cell response against T. gondii. T. gondii infection induces strong innate immunity manifested by a potent NK cell response (1, 42). No differences in the absolute numbers of CD8+ or γδ T cells between the knockout and wild-type mice were observed. However, the knockout mice exhibited an increased NK cell response. To determine NK cell induction against the parasite in CD4−/− mice, the kinetics of this response was measured. In comparison to wild-type mice, CD4−/− mice had a significantly higher and prolonged NK cell response (Fig. 1a). At day 14 p.i. NK cell numbers in the wild-type mice reverted to background levels. By contrast, CD4−/− mice continued to maintain significantly elevated NK cell levels up to day 21 p.i. T. gondii infection resulted in a minimal increase of CD3+ NK cells (data not shown) in both wild-type and knockout mice, suggesting that the elevated NK population belongs to the CD3− NK cell phenotype. These observations suggest that, in the absence of CD4+ T cells, NK cells may play a compensatory role in the knockout mouse.

To determine the importance of NK cells in the resolution of T. gondii infection in CD4−/− mice, depletion studies were performed. Both knockout and wild-type mice were treated with anti-asialo-GM1 antibody three times a week starting 1 day prior to infection. This antibody has been previously used by us (21) and others for NK cell depletion (9, 29). Moreover, pilot studies were performed in which antibody treatment did not have any effect on any of the T-cell subsets. Only one out of six wild-type mice succumbed to early T. gondii infection (Fig. 1b). Depletion of NK cells did not significantly change the outcome of infection. Compared to wild-type mice, none of the CD4−/− mice treated with control antibody showed any mortality due to T. gondii infection. This is an expected response, as previous studies have demonstrated that the absence of CD4+ T cells can cause a reduction in hyperinflammatory response, which ultimately leads to survival of the host (25). Interestingly, 100% of CD4−/− mice depleted of NK cells succumbed to infection and all the animals were dead by day 16 postinfection (Fig. 1b).

To establish that NK cell depletion causes increased parasite multiplication in CD4−/− mice, tissues (spleen, liver, and brain) were analyzed for parasite load by quantitative PCR (23). NK cell depletion caused a significant increase in the parasite DNA in the spleen and liver of CD4−/− mice. In contrast anti-asialo-GM1 antibody treatment did not significantly alter the parasite load in spleen and liver of wild-type mice (Fig. 1c, i and ii). As previously reported by our laboratory (5), brains of CD4−/− mice have higher parasite burden, which is not altered by NK cell depletion (Fig. 1c, iii).

Depletion of NK cells leads to poor CD8+ T-cell immunity in CD4−/− mice. Our previous studies showed that normal CD8+ T-cell immunity can be induced in the absence of CD4+ T cells (5) and that mice lacking CD4+ T cells can survive acute T. gondii infection. However, present studies demonstrate that, unlike wild-type mice, depletion of NK cells in CD4−/− mice results in a higher parasite load and leads to a fatal outcome. To determine if depletion of NK cells has an effect on the development of CD8+ T-cell immunity, phenotypic analysis for activation markers was performed on splenocytes and mesenteric lymph nodes (MLNs) at day 7 p.i. CD8+ T cells were evaluated for the expression of activation markers by dual immunofluorescence. CD69 (a marker for early T-cell activation) (38), was induced in the CD8+ T cells from both wild-type and knockout mice (Fig. 2a). However, CD69 expression on CD8+ T cells was significantly reduced by anti-asialo-GM1 treatment in CD4−/− mice. Similarly CD44 and CD62L expression, characteristic of activated/memory T cells (17, 45), was significantly reduced on the CD8+ T cells of CD4−/− mice administered anti-asialo-GM1 antibody (P = 0.05 and P = 0.002; Fig. 2b and c). Phenotypic analysis of MLNs revealed that CD8+ T-cell activation in the CD4−/− mice infected with T. gondii is hampered due to NK cell de-
In the absence of NK cells, CD8^+ T cells from MLNs of CD4^-/- mice have significantly decreased CD69^+ CD44^hi CD62^low cells.

To evaluate the role of NK cells in the development CD8^+ T-cell immunity against T. gondii in CD4^-/- mice, we assessed antigen-specific CD8^+ T cells in a pCTL assay at day 14 p.i. Depletion of NK cells led to significant reduction in the frequency of antigen-specific CD8^+ T cells in CD4^-/- mice (P = 0.002; Fig. 3a, i). Conversely, pCTL levels were not affected when NK cell depletion was carried out in wild-type mice (Fig. 3a, ii).
In addition to their cytolytic activity against infected targets, activated CD8$^+$ T cells from T. gondii-infected mice also secrete IFN-$\gamma$ (18). The activated-CD8$^+$ T-cell profile in NK cell-depleted mice was further evaluated by estimating the number of IFN-$\gamma$-producing CD8$^+$ T cells. On day 14 p.i., the mice were killed and the CD8$^+$ T-cell population was analyzed for IFN-$\gamma$ production by intracellular staining. As expected, T. gondii infection significantly increased the frequency of IFN-$\gamma$-producing CD8$^+$ T cells in the CD4$^+$ mice ($P = 0.04$) (Fig. 3b). However, when these animals were depleted of NK cells, the percentage of IFN-$\gamma$-producing CD8$^+$ T cells was significantly reduced ($P = 0.03$) and reached background levels. Similar to CD4$^+$ mice, T. gondii infection led to increased IFN-$\gamma$-producing CD8$^+$ T cells, which unlike the knockout mice were not affected by NK cell depletion (data not shown).

**Prolonged IL-12 response in CD4$^-/-$ mice.** Next we evaluated the mechanism for accelerated and prolonged NK cell responses in CD4$^-/-$ mice. Previous studies from our group and other laboratories (9, 10, 21) have demonstrated that strong NK cell responses against T. gondii are dependent on the induction of rapid and strong host IL-12 production (9, 13). This not only leads to robust NK cell response but also polar-

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**FIG. 2.** Phenotypic changes on CD8$^+$ T cells from spleens and MLNs following T. gondii infection. CD4$^-/-$ mice and control littermates were infected perorally with 20 cysts. Groups of mice from both the knockout and wild-type (WT) mice were administered anti-asialo-GM1 antibody as mentioned in the legend for Fig. 1. At day 7 p.i., splenocytes (a to c) and mesenteric lymphocytes (d to f) were isolated. The tissues were pooled (three mice per group), and cells were dual-fluorescence stained with anti-CD8-FITC and either anti-CD44-PE, anti-CD69-PE, or anti-CD62L-PE (PharMingen). Cells were gated for FITC and PE fluorescence. Data are shown as the means of three replicates. The experiment was performed twice with similar results, and data are representative of one experiment.
izes CD4+ T cells towards production of Th1 cytokines like IFN-γ (44). To prevent hyperinflammatory responses in the host, there exists an IL-10-dependent feedback mechanism that interrupts continuous IL-12 release and ultimately prevents overproduction of IFN-γ (11). However, in the absence of CD4+ T cells, prolonged IL-12 production may be needed to maintain a strong NK cell response against T. gondii infection. To determine if this is the case, we measured serum IL-12 and IL-10 levels at various time points postinfection. Strong IL-12 levels are present in the sera of both wild-type and knockout animals at day 3 p.i. (Fig. 4a). However, compared to knockout mice, serum IL-12 levels are significantly reduced in the wild-type mice starting at day 5 p.i. (P = 0.03). IL-12 levels of the knockout mice stay elevated at day 10 p.i., while in the wild-type mice they reach background levels at this time point. In contrast to IL-12, suboptimal levels of IL-10 were detected in the sera of knockout mice. Levels for this cytokine in the knockout mice were significantly reduced compared to wild-type mice at days 5 (P = 0.02) and 7 (P = 0.003) p.i. (Fig. 4b).

To determine if prolonged IL-12 production is critical for survival of CD4+−/− mice, depletion studies were performed. Knockout and wild-type mice were infected perorally with 20 cysts. Anti-IL-12 antibody administration in one group of animals was started at day 0 and continued on days 1 and 2 p.i. (early depletion). In another group the treatment was performed on days 3, 4, and 5 p.i. (late depletion). Previous studies from our laboratory have shown that IL-12 depletion in T. gondii-infected animals is effective only when antibody treatment is started at the time of challenge (21). In these studies, injection of anti-IL-12 antibody at later stages like day 3 p.i. did not alter the outcome of infection. As observed earlier, in the present study, wild-type mice treated with anti-IL-12 antibody starting day 0 p.i. did not show a prolonged NK cell response in IL-12-depleted animals. The above studies suggested that continued IL-12 production is important for survival of CD4+−/− mice against T. gondii infection.

Although the role of IL-12 is considered to be primarily restricted to the induction of IFN-γ production by NK cells (32), there are studies which demonstrate its involvement in proliferation of these cells (24). To determine if neutralization of IL-12 leads to reduction of the NK cell response during T. gondii infection, phenotypic analysis on anti-IL-12-treated knockout mice was performed at day 6 p.i. Anti IL-12 treatment of CD4+−/− mice results in significant (P = 0.013) reduction in the absolute number of NK cells in the spleens of knockout mice (Fig. 5b).

Adoptive transfer of NK cells restores CD8+ T-cell immunity in IL-12-depleted animals. The above studies suggested that a prolonged NK cell response in CD4+−/− mice is dependent on continued IL-12 production, which is critical for induction of robust CD8+ T-cell immunity and ultimate survival of the host. Next we determined if adoptive transfer of NK cells to IL-12-depleted animals can restore CD8+ T-cell immune response against the parasite and ensure its survival. The knockout animals were infected with T. gondii cysts and de-
pleted of IL-12 as described above. The animals were injected intravenously with $10^7$ purified (99% pure) NK cells either from syngeneic wild-type or IFN-γ/H9253/H11002 mice at day 6 p.i. The CD4+/H11001 donors who received NK cells from IFN-γ/H9253/H11002 mice did not survive significantly longer than the controls, which did not receive any cells (Fig. 6a). Conversely, 50% of the knockout recipients that were injected with NK cells from the naive wild-type mice were able to survive infection until the termination of the experiment (Fig. 6a).

To further determine the effect of NK cell transfer on CD8+ T-cell response of the recipients, pCTL analysis was conducted. CD8+ T cells from the IL-12-depleted recipients were isolated at day 12 p.i. and cultured by limiting dilution and an assay was performed. Similar to survival data, the CD8+ T cells from knockout recipients injected with NK cells from wild-type donors exhibited high pCTL frequency (1/2,666) (Fig. 6b ii), which was comparable to control knockout mice which were not treated with anti-IL-12 antibody. Conversely, IL-12-depleted knockout recipients treated with NK cells from IFN-γ−/− donors had significantly lower ($P = 3.5 \times 10^{-4}$) pCTL frequency within their CD8+ T-cell population.

**DISCUSSION**

We now show that, in the absence of CD4+ T cells, NK cells play a pivotal role in the induction of CD8+ T-cell immunity against Toxoplasma gondii. Mice lacking CD4+ T cells have strong and prolonged NK cell responses. NK cell depletion severely impairs CD8+ T-cell immunity in CD4−/− animals without having any effect on wild-type mice. Upregulation of NK cell responses in the knockout animals is dependent on continued IL-12 production. Anti-IL-12 treatment in the knockout animals reduces NK cell responses that lead to poor CD8+ T-cell immunity. These findings have implications for immunocompromised individuals with defective CD4+ T-cell immunity who may generate poor CD8+ T-cell immunity against intracellular infections or cancers.
Both CD4⁺ and CD8⁺ T cells play an important role in the resolution of T. gondii infection (8, 41). However, it is the CD8⁺ T cells that act as primary effectors, with CD4⁺ T cells playing a synergistic role (5, 8, 18). Previous studies from our laboratory have reported that, although CD4⁺ T cells are important for maintaining long-term CD8⁺ T-cell immunity against the parasite, a primary CD8⁺ T-cell response against T. gondii in mice lacking conventional CD4⁺ T cells can be induced (5). In present studies for the first time we demonstrate that CD4⁻/⁻ mice develop a prolonged NK cell response, which is responsible for the induction of primary CD8⁺ T-cell immunity. Depletion of NK cells in the CD4⁻/⁻ mice down-regulates the CD8⁺ T-cell response against the parasite, and animals become susceptible to infection. Earlier studies by Denkers et al. have shown that major histocompatibility complex class II-deficient mice, which lack CD4⁺ T cells, develop an IL-2-producing CD4⁺ NK1.1 response, which is involved in priming CD8⁺ T-cell immunity against T. gondii (6). However, as stated in Results we did not see any increase in CD3⁺ NK1.1 cells in the mice lacking conventional CD4⁺ T cells. As these mice lack the CD4⁺ gene, the absence of CD4⁺ NK1.1 cells in these animals is not surprising. Nor did we see an emergence or increase of CD3⁻ double-negative cells in response to infection (data not shown), as reported by Locksley et al. (26) in a Leishmania major model. Thus our data convincingly prove that induction of CD8⁺ T-cell immunity is dependent on increased and prolonged CD3⁻ NK cell numbers in these animals, which has not been so far described in an infectious disease model. High NK cell numbers can be attributed to longer duration of IL-12 production. The importance of IL-12 in the protective immunity against T. gondii is well established by us (21) and others (3, 9). Previous studies from our laboratory have shown that, while depletion of IL-12 at the start of infection has a lethal outcome for immunocompetent animals infected with T. gondii, delayed anti-IL-12 treatment has no effect on the survival of these animals (21). Our present studies confirm these findings. However, unlike wild-type mice, mortality in CD4⁻/⁻ mice was observed even when anti-IL-12 treatment began late (3 days after infection). These results suggest that prolonged IL-12 release is critical for the NK cell responses, which are important for priming CD8⁺ T-cell immunity against the pathogen. In addition to increased inflammatory cytokine IL-12 in CD4⁻/⁻ mice, there is an obvious absence of IL-10 in these animals. IL-10 is a down-regulatory cytokine that plays an important role in the regulation of hyperinflammation in T. gondii-infected immunocompetent animals (11, 14). IL-10 response during T. gondii infection is essential to down-regulate the levels of IFN-γ to prevent pathology in the host (11). However, as CD4⁺ T cells are major IFN-γ producers during acute T. gondii infection (25), the chances of hyperinflammation as a result of T. gondii infection in the knockout animals is minimal. Hence, down-regulatory cytokines like IL-10, which have important anti-inflammatory roles in a normal situation, are not needed. As a matter of fact, continued IL-12 production is very important for maintenance of robust NK cell immunity. NK cells are the primary source of IFN-γ production in this situation, which is needed for induction of optimal CD8⁺ T-cell response against the pathogen.

Recent studies have focused on the generation of CD4⁺-dependent and -independent CD8⁺ T-cell immunity against microbial infections (39) (36, 40). While the presence of CD4⁺ T cells is critical for the development of CD8⁺ T-cell immunity, generation of efficient CD8⁺ T-cell immunity in the absence of CD4⁺ T-cell help has been noted during infection with other infectious agents (30, 33). Previous studies from our laboratory have shown that optimal CD8⁺ T-cell immunity against T. gondii infection in CD4⁺ T-cell-deficient mice can be induced (5). Although CD4⁺-independent CD8⁺ T-cell immunity has been described in other infectious diseases, the mechanisms of this response are not understood. In the present studies we report that the primary CD8⁺ T-cell response against T. gondii infection in the absence of CD4⁺ T-cell help is dependent on CD3⁻ NK cells. Depletion of NK cells in the
infected CD4-deficient mice causes poor CD8+ T-cell activation resulting in the reduction of antigen-specific cytotoxicity.

IL-12 is important in the induction of a strong NK cell response, and treatment with anti-IL-12 even at the later stages of infection causes mortality in CD4−/− mice. Although the importance of IL-12 is related to the induction of IFN-γ production by NK cells (32), our present studies demonstrate that during T. gondii infection IL-12 also plays a role in NK cell proliferation. Adoptive transfer of IL-12-depleted mice with NK cells from normal immunocompetent mice caused significant reduction in their mortality and restored their antigen-specific CD8+ T-cell immunity. Although accentuated NK cell responses can enhance protection against T. gondii (9), the ultimate survival of the animals is dependent on T cells (15). Thus, it can be concluded that long-term survival of IL-12-depleted recipients is due to enhanced CD8+ T-cell immunity generated as a result of NK cell transfer. Our study for the first time demonstrates the direct role of CD3− NK cells in the priming of CD8+ T-cell immunity against an intracellular pathogen, in the absence of CD4+ T-cell help.

Thus the importance of NK cells in CD4−/− mice is not restricted to their ability to protect these mice against acute infection (10). IFN-γ-producing NK cells in the normal immunocompetent host, while important (12), may not be essential for host survival against T. gondii challenge. Based on our findings, in the absence of CD4+ T-cell help these cells are essential for the development of CD8+ T-cell immunity against the parasite. As a consequence of reduced NK cells, CD4−/− mice are unable to develop a robust CD8+ T-cell response. Adoptive transfer of NK cells to these mice reduces mortality and also restores their CD8+ T-cell immunity.

The mechanism of NK cell-induced CD8+ T-cell response is dependent on IFN-γ, as donor NK cells from IFN-γ−/− mice fail to restore CD8+ T-cell immunity in IL-12-depleted knockout animals. Our findings are well supported by recent observations demonstrating that, due to their ability to secrete IFN-γ, NK cells can prime dendritic cells to induce a protective CD8+ T-cell response (29). Based on these studies we put forward the following hypothesis. T. gondii infection induces strong inflammatory cytokine production by NK and CD4+ T cells. Under normal conditions, IFN-γ-producing CD4+ T cells provide help for the priming of the CD8+ T-cell response. However, in the absence of these cells, NK cells, due to their ability to produce IFN-γ, take over the role of providing necessary help to CD8+ T cells. Depletion of NK cells in these animals down-regulates CD8+ T response and compromises the protective immunity against the parasite. It has been observed that reactivation of Toxoplasma infection in human immunodeficiency virus-infected individuals occurs during full-blown AIDS, when, as a consequence of reduced CD4+ T-cell immunity, there is also a decline in CD8+ T cells (35). Similarly, using the murine AIDS model, our laboratory has demonstrated that reactivation of latent Toxoplasma infection in the host can be prevented by adoptively transferring immune CD8+ T cells to these mice (19). Thus, maintenance of adequate CD8+ T-cell immunity appears to be critical for combating latent T. gondii reactivation. It will be very interesting to determine if, in the absence of sufficient CD4+ T cells, acceleration of NK cell response in these and other immunocompromised hosts can help to maintain CD8+ T-cell immunity against T. gondii and keep chronic infection under control.

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